AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Amended) A method of making a contrast agent having resistance against collapse from pressure increases when used in ultrasonic echography, said contrast agent consisting of gas-filled microvesicles suspended in an aqueous liquid carrier phase, the microvesicles being [either] microbubbles filled with a physiologically acceptable gas wherein the gas is bounded by a stabilizing layer of one or more film forming phospholipids in lamellar or laminar form at the gas/liquid interface [bounded by an evanescent gas/liquid interfacial closed surface, or microballoons bounded by a material envelope], said method comprising the step of forming the microvesicles in the presence of the [a physiologically acceptable gas, or gas mixture comprising a physiologically acceptable gas, or filling preformed microvesicles with said gas, or gas mixture , said] physiologically acceptable gas [being] selected from the group consisting of SF_6, [SeF_6,] CF_4, $CBrF_3$, C_4F_8, $CClF_3$, [CCl_2F_2,], C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$, [CBr_2F_2] and C_4F_{10}, said microvesicles having resistance against collapse resulting, at least in part, from pressure increases effective when a suspension of said gas-filled microvesicles is [in] injected into the bloodstream of a patient.

2. (Amended) A method of making a contrast agent having resistance against collapse from pressure increases when used in ultrasonic echography, said contrast agent consisting of gas-filled microvesicles suspended in an aqueous liquid [carder] carrier phase, the microvesicles being [either] microbubbles filled with a physiologically acceptable gas wherein the gas is bounded by a stabilizing layer of one or more film forming phospholipids in lamellar or laminar form at the gas/liquid interface [bounded by an evanescent gas/liquid interfacial closed surface, or microballoons bounded by a material envelope], said method comprising the steps of:

preforming the microvesicles or precursors thereof under an atmosphere of a first

gas; and

substantially substituting at least a fraction of said first gas with a second gas which is [a physiologically acceptable gas, or gas mixture comprising a physiologically acceptable gas, said] the physiologically acceptable gas [being] selected from the group consisting of SF_6, [SeF_6,] CF_4, $CBrF_3$, C_4F_8, $CClF_3$, [CCl_2F_2,] C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$, [CBr_2F_2,] and C_4F_{10}, said microvesicles having resistance against collapse resulting, at least in part, from pressure increases effective when a suspension of said gas-filled microvesicles is injected into the bloodstream of a patient.

3. The method of claim 2, in which the gas used in the first step allows effective control of the average size and concentration of the microvesicles in the carrier liquid, and the physiologically acceptable gas added in the second step ensures prolonged useful echogenic life of the suspension for in-vivo ultrasonic imaging.

4.-6. Cancelled.

7. (Amended) The method of claim [5] 1, in which [in] at least one of the phospholipids is a diacylphosphatidyl compound wherein the acyl group is a C_{16} fatty acid residue or a higher homologue thereof.

8.-12. Cancelled.

13. (Amended) A method of making a contrast agent for ultrasonic echography which consists of gas-filled microbubbles [microvesicles] suspended in an aqueous liquid carrier phase, the microbubbles [microvesicles] having resistance against collapse resulting from pressure increases effective when the said suspensions are injected into the bloodstream of a patient, and the microbubbles being filled with a physiologically acceptable gas wherein the gas is bounded by a stabilizing layer of one or more film forming phospholipids in lamellar or laminar form at the gas/liquid interface, said method comprising the step of forming the microbubbles [microvesicles] in the presence of [a physiologically acceptable gas or gas mixture comprising a physiologically acceptable gas, or filling preformed microvesicles with said gas or said gas mixture,] said physiologically acceptable gas [being] selected from the group SF_6, [SeF_6,] CF_4, $CBrF_3$,

C_4F_8, $CClF_3$, [CCl_2F_2,] C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$, [CBr_2F_2] and C_4F_{10}, said gas [or at least a gas in said gas mixture] being such that, under standard conditions, the pressure difference ΔP between pressures at which the bubble counts are about 75% and 25% of the original bubble count is at least 25 Torr.

14. An aqueous suspension made according to the method of claim 13, wherein the physiologically acceptable gas is such that, under standard conditions, and at a rate of pressure increase to the suspension of about 100 Torr/min, the pressure difference ΔP between pressures at which the bubble counts are about 75% and 25% of the original bubble count is at least 25 Torr.

15. A method of making a contrast agent having resistance against collapse from pressure increases when used in ultrasonic echography, said contrast agent consisting of gas-filled microvesicles suspended in an aqueous liquid carrier phase, the microvesicles being microbubbles filled with a gas mixture wherein the gas mixture is bounded by a stabilizing layer of one or more film forming phospholipids in lamellar or laminar form at the gas/liquid interface, said method comprising the step of forming the microvesicles in the presence of the gas mixture comprising a physiologically acceptable gas, selected from the group consisting of SF_6, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$ and C_4F_{10}, said microvesicles having resistance against collapse resulting, at least in part, from pressure increases effective when a suspension of said gas-filled microvesicles is injected into the bloodstream of a patient.

16. A method of making a contrast agent having resistance against collapse from pressure increases when used in ultrasonic echography, said contrast agent consisting of gas-filled microvesicles suspended in an aqueous liquid carrier phase, the microvesicles being microballoons consisting of a physiologically acceptable gas bounded by an organic polymer envelope at the gas/liquid interface, said polymer envelope formed from one or more polymers selected from the group consisting of polylactic or polyglycolic acid and their copolymers, denatured albumin, reticulated hemoglobin, and esters of polyglutamic and polyaspartic acids, said method comprising the step of forming the

microvesicles in the presence of said physiologically acceptable gas selected from the group consisting of SF_6, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$ and C_4F_{10}, said microvesicles having resistance against collapse resulting, at least in part, from pressure increases effective when a suspension of said gas-filled microvesicles is injected into the bloodstream of a patient.

17. A method of making a contrast agent having resistance against collapse from pressure increases when used in ultrasonic echography, said contrast agent consisting of gas-filled microvesicles suspended in an aqueous liquid carrier phase, the microvesicles being microballoons consisting of a gas mixture bounded by an organic polymer envelope at the gas/liquid interface, said polymer envelope formed from one or more polymers selected from the group consisting of polylactic or polyglycolic acid and their copolymers, denatured albumin, reticulated hemoglobin, and esters of polyglutamic and polyaspartic acids, said method comprising the step of forming the microvesicles in the presence of the gas mixture comprising a physiologically acceptable gas, selected from the group consisting of SF_6, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$ and C_4F_{10}, said microvesicles having resistance against collapse resulting, at least in part, from pressure increases effective when a suspension of said gas-filled microvesicles is injected into the bloodstream of a patient.

18. A method of making a contrast agent having resistance against collapse from pressure increases when used in ultrasonic echography, said contrast agent consisting of gas-filled microvesicles suspended in an aqueous liquid carrier phase, the microvesicles being microbubbles filled with a gas mixture wherein the gas mixture is bounded by a stabilizing layer of one or more film forming phospholipids in lamellar or laminar form at the gas/liquid interface, said method comprising the steps of:

preforming the microvesicles or precursors thereof under an atmosphere of a first gas; and

substantially substituting at least a fraction of said first gas with a second gas which is the gas mixture comprising a physiologically acceptable gas selected from the

group consisting of SF_6, CF_4, $CBrF_3$, C_4F_8, $CC1F_3$, C_2F_6, C_2C1F_5, $CBrClF_2$, $C_2C1_2F_4$ and C_4F_{10}, said microvesicles having resistance against collapse resulting, at least in part, from pressure increases effective when a suspension of said gas-filled microvesicles is injected into the bloodstream of a patient.

19. A method of making a contrast agent having resistance against collapse from pressure increases when used in ultrasonic echography, said contrast agent consisting of gas-filled microvesicles suspended in an aqueous liquid carrier phase, the microvesicles being microballoons consisting of a physiologically acceptable gas bounded by an organic polymer envelope at the gas/liquid interface, said polymer envelope formed from one or more polymers selected from the group consisting of polylactic or polyglycolic acid and their copolymers, denatured albumin, reticulated hemoglobin, and esters of polyglutamic and polyaspartic acids, said method comprising the steps of:

preforming the microvesicles or precursors thereof under an atmosphere of a first gas; and

substantially substituting at least a fraction of said first gas with a second gas which is the physiologically acceptable gas selected from the group consisting of SF_6, CF_4, $CBrF_3$, C_4F_8, $CC1F_3$, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2C1_2F_4$ and C_4F_{10}, said microvesicles having resistance against collapse resulting, at least in part, from pressure increases effective when a suspension of said gas-filled microvesicles is injected into the bloodstream of a patient.

20. A method of making a contrast agent having resistance against collapse from pressure increases when used in ultrasonic echography, said contrast agent consisting of gas-filled microvesicles suspended in an aqueous liquid carrier phase, the microvesicles being microballoons consisting of a gas mixture bounded by an organic polymer envelope at the gas/liquid interface, said polymer envelope formed from one or more polymers selected from the group consisting of polylactic or polyglycolic acid and their copolymers, denatured albumin, reticulated hemoglobin, and esters of polyglutamic and polyaspartic acids, said method comprising the steps of

preforming the microvesicles or precursors thereof under an atmosphere of a first gas; and

substantially substituting at least a fraction of said first gas with a second gas which is the gas mixture comprising a physiologically acceptable gas selected from the group consisting of SF_6, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$ and C_4F_{10}, said microvesicles having resistance against collapse resulting, at least in part, from pressure increases effective when a suspension of said gas-filled microvesicles is injected into the bloodstream of a patient.

21. The method of claim 18, in which the gas used in the first step allows effective control of the average size and concentration of the microvesicles in the carrier liquid, and the physiologically acceptable gas added in the second step ensures prolonged useful echogenic life of the suspension for in-vivo ultrasonic imaging.

22. The method of claims 19 or 20, in which the gas used in the first step allows effective control of the average size and concentration of the microvesicles in the carrier liquid, and the physiologically acceptable gas added in the second step ensures prolonged useful echogenic life of the suspension for in-vivo ultrasonic imaging.

23.-25. Cancelled.

26. The method of claim 15, in which at least one of the phospholipids is a diacylphosphatidyl compound wherein the acyl group is a C_{16} fatty acid residue or a higher homologue thereof.

27.-29. Cancelled.

30. The method of claims 16 or 17, in which the forming of vesicles with said physiologically acceptable gas is effected by alternately subjecting dry precursors thereof to reduced pressure and restoring the pressure with said gas, and dispersing the precursors in a liquid carrier.

31. The method of claims 16 or 17, in which the filling of the microballoons with said physiologically acceptable gas is effected by flushing the suspension with said gas under ambient pressure.

32. A method of making a contrast agent for ultrasonic echography which consists of gas-filled microbubbles suspended in an aqueous liquid carrier phase, the microbubbles having resistance against collapse resulting from pressure increases effective when the said suspensions are injected into the bloodstream of a patient and the microbubbles being filled with a gas mixture wherein the gas mixture is bounded by a stabilizing layer of one or more film forming phospholipids in lamellar or laminar form at the gas/liquid interface, said method comprising the step of forming the microbubbles in the presence of the gas mixture comprising a physiologically acceptable gas selected from the group consisting of SF_6, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2C1_2F_4$ and C_4F_{10}, said gas or at least a gas in said gas mixture being such that, under standard conditions, the pressure difference ΔP between pressures at which the bubble counts are about 75 % and 25 % of the original bubble count is at least 25 Torr.

33. A method of making a contrast agent for ultrasonic echography which consists of gas-filled microballoons suspended in an aqueous liquid carrier phase, the microballoons having resistance against collapse resulting from pressure increases effective when the said suspensions are injected into the bloodstream of a patient and the microballoons consisting of a physiologically acceptable gas bounded by an organic polymer envelope at the gas/liquid interface, said polymer envelope formed from one or more polymers selected from the group consisting of polylactic or polyglycolic acid and their copolymers, denatured albumin, reticulated hemoglobin, and esters of polyglutamic and polyaspartic acids, said method comprising the step of forming the microballoons in the presence of the physiologically acceptable gas selected from the group consisting of SF_6, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2C1_2F_4$ and C_4F_{10}, said gas or at least a gas in said gas mixture being such that, under standard conditions, the pressure difference ΔP between pressures at which the bubble counts are about 75 % and 25 % of the original bubble count is at least 25 Torr.

34. A method of making a contrast agent for ultrasonic echography which consists of gas-filled microballoons suspended in an aqueous liquid carrier phase, the

microballoons having resistance against collapse resulting from pressure increases effective when the said suspensions are injected into the bloodstream of a patient and the microballoons consisting of a gas mixture bounded by an organic polymer envelope at the gas/liquid interface, said polymer envelope formed from one or more polymers selected from the group consisting of polylactic or polyglycolic acid and their copolymers, denatured albumin, reticulated hemoglobin, and esters of polyglutamic and polyaspartic acids, said method comprising the step of forming the microballoons in the presence of the gas mixture comprising a physiologically acceptable gas selected from the group consisting of SF_6, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$ and C_4F_{10}, said gas or at least a gas in said gas mixture being such that, under standard conditions, the pressure difference ΔP between pressures at which the bubble counts are about 75% and 25 % of the original bubble count is at least 25 Torr.

35. An aqueous suspension made according to the method of claim 32, wherein the physiologically acceptable gas is such that, under standard conditions, and at a rate of pressure increase to the suspension of about 100 Torr/min, the pressure difference ΔP between pressures at which the bubble counts are about 75% and 25% of the original bubble count is at least 25 Torr.

36. An aqueous suspension made according to the method of claims 33 or 34, wherein the physiologically acceptable gas is such that, under standard conditions, and at a rate of pressure increase to the suspension of about 100 Torr/min, the pressure difference ΔP between pressures at which the bubble counts are about 75% and 25% of the original bubble count is at least 25 Torr.

37. The method of claims 1 or 15, wherein the physiologically acceptable gas is selected from the group consisting of CF_4, C_2F_6, C_4F_8, or C_4F_{10}.

38. The method of claim 1, wherein the physiologically acceptable gas is CF_4.

39. The method of claim 1, wherein the physiologically acceptable gas is C_2F_6.

40. The method of claim 1, wherein the physiologically acceptable gas is C_4F_8.

41. The method of claim 1, wherein the physiologically acceptable gas is C_4F_{10}.

42. The method of claim 1, wherein the physiologically acceptable gas is SF_6.

43. The method of claims 16 or 17, wherein the physiologically acceptable gas is selected from the group consisting of CF_4, C_2F_6, C_4F_8, or C_4F_{10}.

44. The method of claim 16, wherein the physiologically acceptable gas is CF_4.

45. The method of claim 16, wherein the physiologically acceptable gas is C_2F_6.

46. The method of claim 16, wherein the physiologically acceptable gas is C_4F_8.

47. The method of claim 16, wherein the physiologically acceptable gas is C_4F_{10}.

48. The method of claim 16, wherein the physiologically acceptable gas is SF_6.

49. The method of claim 1, in which at least part of the phospholipids are in the form of liposomes.

50. The method of claim 15, in which at least part of the phospholipids are in the form of liposomes.